HANDBOOK OF NEUROTOXICOLOGY

Volume I

Edited by

EDWARD J. MASSARO

The National Health and Environmental Effects Research Laboratory, Research Triangle Park, Durham, NC

HUMANA PRESS
TOTOWA, NEW JERSEY

26
Nemertine Toxins

William R. Kem

1. INTRODUCTION

In 1936 a Belgian pharmacologist reported the serendipitous discovery of at least two different toxins in nemertines, a relatively small phylum of marine worms. Bacq demonstrated that an aqueous homogenate of the hoplonemertine *Amphiporus lactifloreus* potently contracted isolated frog skeletal muscle and stimulated the cat cervical autonomic ganglion in a manner similar to the neurotransmitter acetylcholine (ACh). However, since the this activity was stable in highly alkaline solution, it could not be due to ACh *(1,2)*. In other nemertine extracts Bacq also found a neurotoxic activity lacking nicotinic-receptor effects, which he referred to as "nemertine." Both "amphiporine" and "nemertine" extracts caused convulsions, paralysis, and death when injected into crabs. In contrast with "amphiporine" activity, "nemertine" activity only slowly traversed a dialysis membrane. Harold King, an organic chemist who had previously determined the structures of a variety of plant natural products, including the arrow poison d-tubocurarine, attempted crystallization of the active constituent from an extract of 1000 worms. Although this was unsuccessful, the solubility of "amphiporine" activity in chloroform under basic but not acidic conditions indicated that it was a weakly basic compound *(3)*. This was also consistent with Bacq's inference that "amphiporine" was an alkaloid similar to nicotine.

Thirty years elapsed before nemertine toxins were investigated again. During the intervening decades, many new isolation and analytical techniques had been introduced. These included chromatographic methods permitting isolation of even minute amounts of natural products, nuclear magnetic resonance (NMR) and mass spectroscopic techniques, and for peptides and proteins, sensitive amino acid analysis, and Edman sequencing methods. The author, as a graduate student, isolated the hoplonemertine alkaloid anabaseine, a nicotinoid compound possessing a biological and chemical profile similar to Bacq's "amphiporine." Related compounds were found in other hoplonemertines *(4–8)*. In contrast, anoplan (physically unarmed) nemertines were found to contain peptide neurotoxins resembling the "nemertine" activity profile, thus explaining Bacq's observation of a slow rate of "nemertine" activity dialysis *(9)*.

While almost 900 species of nemertines have already been described in the biological literature, it is almost certain that this relatively inconspicuous animal phylum con-

From: Handbook of Neurotoxicology, vol. 1
Edited by: E. J. Massaro © Humana Press Inc., Totowa, NJ

tains many more, as yet undescribed species, perhaps several times this number. While no nemertine fossils have been reported, this group of marine animals is thought to have evolved from the flatworms (Phylum Platyhelminthes) back in Precambrian times (>500 million years ago). Nemertines possess several evolutionary innovations relative to flatworms, including separation of sexes (they are dioecious rather than hermaphroditic), a closed circulatory system composed of pulsating blood vessels, and a unidirectional gastrointestinal system allowing digested food to be eliminated through an anus rather than by mouth *(10)*. They also possess another important structure, namely a long mobile proboscis, which is primarily used to capture prey (Fig. 1A). The phylum is divided into two large systematic classes, based on whether the proboscis is armed (Fig. 1B) with a skin-puncturing stylet or is "unarmed." The armed nemertines are called hoplonemertines, whereas the unarmed species are either paleonemertines (a group thought to represent a more primitive stage in nemertine evolution) or heteronemertines. While the hoplonemertines paralyze their prey (usually other worms or cructaceans, depending on the species), paleonemertine and heteronemertine toxins are likely to be used for defensive purposes only. Since the integuments of hoplonemertines as well as paleonemertines and heteronemertines contain toxins to repel predators, this suggests that they originated for defensive purposes, but in the case of hoplonemertines also became offensive toxins for prey capture.

This chapter will mainly focus on the chemical and pharmacological properties of the few toxins that have been isolated and characterized until now. Their mechanisms of action, insofar as they are known, will then be described. Finally, we will consider the potential utility of the toxins as neurobiological research tools and models for drug design.

2. HOPLONEMERTINE TOXINS

A plethora of alkaloids have been discovered in hoplonemertines, but only a small number of these compounds have as yet been isolated and studied *(4–9)*. The major problem is one of collecting and identifying satisfactory amounts of biomass from which the compounds can be extracted in sufficient quantity for structural identification. In this section we will only describe compounds whose structures have been previously reported.

2.1. Anabaseine

2.1.1. Chemistry

Anabaseine (Fig. 2) was first isolated from the Peregrine Hoplonemertine *Paranemertes peregrina (4,5)*. This moderately large (length > 15 cm) species wanders over exposed surfaces at low tide, searching for its annelid prey in full view of potential predators. Anabaseine was later found in certain ant venoms *(11)*. While the structure of anabaseine chemically resembles anabasine, a tobacco alkaloid, it differs from the latter compound in one important chemical bond: there is a double bond between the nitrogen atom in the otherwise saturated ring and the carbon atom, which also is connected to the pyridyl ring. An imine-enamine tautomerism makes the tetrahydropyridyl ring beta-carbon lie within the same plane as this alpha-carbon and the imine N. This system, in turn, is conjugated with the pi electrons of the pyridyl ring. There-

A

Fig. 1. (A) potential pred prey, an amphi along the Pac hoplonemertin used to punctu glandular epith enter the crust and enters the replaced with covering the re boscis and als Another group attacking prey

fore, the two r tine and anab each other in

Anabaseine Austrian toba in a very low more efficien those wishing tection of the groups. Dire

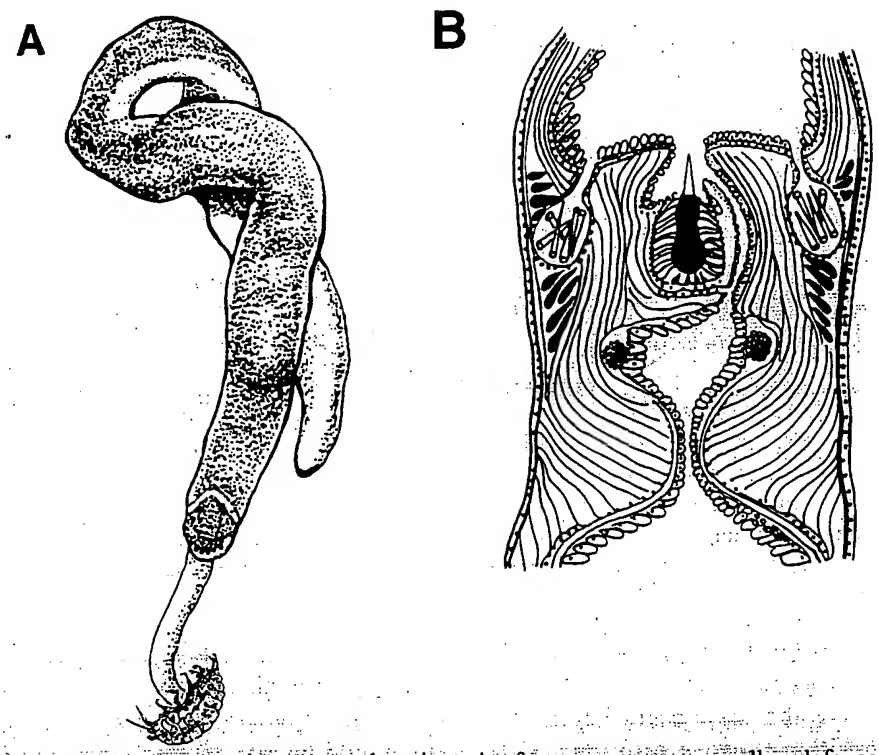


Fig. 1. **(A)** Hoplonemertines use proboscis toxins for prey capture as well as defense against potential predators. **(A)** The Chevron hoplonemertine, *Amphiporus angulatus*, attacking its prey, an amphipod crustacean. This hoplonemertine (maximum length about 10–15 cm) occurs along the Pacific and Atlantic coasts of North America. **(B)** A general diagram of the hoplonemertine median proboscis stylet apparatus. The mineralized stylet of this apparatus is used to puncture the skin of the prey, thus allowing pyridyl alkaloid toxins produced in the glandular epitheliuim of the anterior proboscis and stored in the posterior chambers to readily enter the crustacean. The actual mechanism by which the venom exits the posterior chamber and enters the victim is not yet clear. The stylet is often lost during prey capture, but is readily replaced with another stylet kept in one of the two stylet accessory pouches. The integument covering the rest of the worm is continuous with the secretory epithelium of the anterior proboscis and also produces and secretes toxins used as a chemical defence against predators. Another group of hoplonemertines, the Polystyliferans, possesses multiple stylets for use in attacking prey *(18a)*.

fore, the two rings of anabaseine are approximately coplanar. This contrasts with nicotine and anabasine, whose two rings are approximately at right angles with respect to each other in aqueous solution.

Anabaseine was first obtained as a intermediate in the synthesis of anabasine by two Austrian tobacco chemists *(12)*. This classical method generally provided anabaseine in a very low yield *(5,13)*. Subseqently, several modifications were made to provide a more efficient synthesis and isolation *(14,15)*; these papers should be consulted by those wishing to prepare the compound, which is not commercially available. The protection of the piperidone nitrogen can be accomplished with a variety of chemical groups. Direct crystallization of the ammonium-ketone open-chain form as a

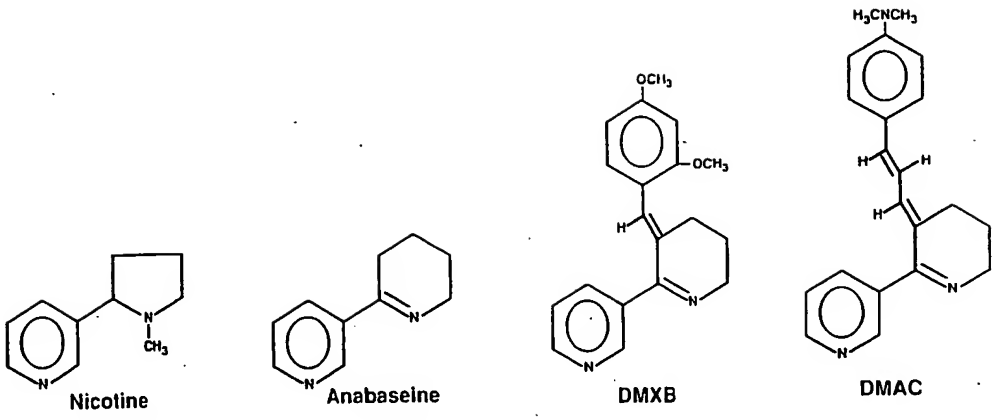


Fig. 2. Covalent structures of nicotine, anabaseine (*Paranemertes* toxin), 3-(2,4-dimethoxybenzylidene)-anabaseine (also called GTS-21 or DMXBA), and DMAC-anabaseine. While both nicotine and anabaseine stimulate most nicotinic receptors, GTS-21 and DMAC-anabaseine only stimulates the α_7-type nicotinic receptors occurring in the mammalian brain. GTS-21 is currently undergoing clinical tests for possible use in treating neurodegenerative diseases *(26,28)*.

dihydrochloride salt from the final reaction was found to be much more efficient than the older workup methods. Synthetic anabaseine dihydrochloride obtained in this manner exists as the ammonium-ketone form. While stable as the dried salt, to avoid any decomposition aqueous solutions of the toxin should be refrigerated when not in use and replaced after 1–2 wk. The cationic forms of anabaseine are quite soluble in protic solvents such as water, methanol, and ethanol, but the less hydrophilic free base is best dissolved in nonaqueous solvents such as alcohols, acetone, or ethyl acetate.

Anabaseine occurs in several different forms in the presence of water *(4)*. An NMR investigation demonstrated that at neutral pH there are three main forms present in roughly equal concentrations *(16)*. These are the free base (cyclic imine), the monocationic cyclic iminium, and the monocationic ammonium-ketone. This multiplicity of forms complicated our initial attempts at identifying the pharmacologically active form that interacts with nicotinic receptors. Thus, stable analogs of these three forms were prepared so that their individual pharmacological properties could be examined. The fully aromatized free-base analog 2,3'-bipyridyl can be expected to possess a chemical conformation similar to the cyclic imine form of anabaseine, while 2-(3,4,5,6-tetrahydropyrimidinyl)-3-pyridine (called PTHP) was selected as an appropriate analog for the cyclic iminium form. To obtain stable open-chain forms of anabaseine, the open-chain nitrogen of anabasine was di- or tri-methylated. Since only PTHP displayed an ability to contract skeletal muscle and to bind to brain nicotinic receptors, we concluded that the mono-protonated cyclic iminium species is the only form of anabaseine that possesses significant affinty for the nicotinic receptor *(17)*.

2.1.2. *Pharmacology*

Anabaseine stimulates a variety of vertebrate nicotinic receptors, like nicotine *(18)*. However, it preferentially stimulates nicotinic receptors, namely skeletal muscle and brain α_7 subtypes, which display high affinities for the snake toxin α-bungarotoxin (Table 1). Nicotine preferentially stimulates other neuronal nicotinic receptors that are

Table 1
Comparison of t
on Several Verte

Receptor type	
Central	
α_7	
(Rat)	
α_4-β_2	
(Rat)	
Peripheral	
Sympathetic	
(Rat PC12)	
Skeletal muscle	
(Frog)	

. Table data summa

involved in its eu
rotoxin nicotinic
tent, when the io
previously establi
muscular receptor

Patch-clamp an
receptors showed
may be considere
the single channel
anabaseine also is

While nicotine
β_2 subunits, anaba
receptors that have
Anabaseine was o
full agonist at the
courses of the ioni
ACh, suggests that
agonists at this rec

Nicotine was a
major brain nicotin
greater than upon t
lower (about 100-f
ing concentrations
receptors.

The whole anim
(6). Because of its l
with anabaseine. M
in nucleus basalis-l

Table 1
Comparison of the Relative Efficacies of Anabaseine, Nicotine, and GTS-21 on Several Vertebrate Nicotinic Receptors

Receptor type	*Anabaseine*	*Nicotine*	*GTS-21*
Central			
α_7 (Rat)	Full agonist	Weak partial agonist	Partial agonist
α_4-β_2 (Rat)	Weak partial agonist	Strong partial agonist	Antagonist
Peripheral			
Sympathetic (Rat PC12)	Full agonist	Full agonist	Weak antagonist
Skeletal muscle (Frog)	Full agonist	Full agonist	Weak antagonist

Table data summarized from results from refs. *18* and *28*.

involved in its euphoric action in the brain. Anabaseine is one of the most potent neurotoxin nicotinic agonists; only epibatidine, anatoxin, and leptodactyline are more potent, when the ionized forms of these compounds are compared (Table 2). It was previously established that the monocationic form of nicotine stimulates the neuromuscular receptor *(19)*.

Patch-clamp analysis of anabaseine action on BC3H cell neuromuscular nicotinic receptors showed that anabaseine's efficacy is comparable with that of ACh; thus it may be considered a full agonist on the neuromuscular type receptor *(18)*. Analysis of the single channel openings provided evidence that at relatively high concentrations, anabaseine also is a channel-blocker (Fig. 3).

While nicotine with high potency stimulates central neuronal receptors containing β_2 subunits, anabaseine displays a relatively low potency for stimulating these central receptors that have been implicated in tobacco addiction as well as cognitive function. Anabaseine was only a weak partial agonist at the rat α_4-β_2 subtype of receptor, but a full agonist at the brain α_7 subunit containing receptor (Fig. 4). The prolonged time-courses of the ionic currents generated by anabaseine or nicotine, relative to that of ACh, suggests that these nicotinoid compounds also act as channel-blockers as well as agonists at this receptor subtype.

Nicotine was a partial agonist (relative to the natural agonist ACh) at both of the major brain nicotinic receptors, but its maximum effect on the α_4-β_2 subtype was much greater than upon the α_7 receptor. Since nicotine also binds to α_4-β_2 receptors at much lower (about 100-fold) concentrations than at α_7 receptors, its in vivo effects at smoking concentrations seem to be mediated primarily through the β_2 subunit-containing receptors.

The whole animal (mouse) toxicity of anabaseine is very similar to that of nicotine *(6)*. Because of its lack of receptor selectivity, few in vivo studies have been carried out with anabaseine. Meyer et al. *(20)* found that anabaseine improved passive avoidance in nucleus basalis-lesioned rats. We found that, when injected into the lateral ventricle

Table 2
Relative Potencies of Nicotinic Agonists on the Frog Rectus Abdominis Muscle[a]

Compound	*EC_{50} (μM)*	*pK_a*	*$EC_{50,I}$ (nM)*	*Relative potency ($EC_{50}Carb/EC_{50,I}$)*
Epibatidine	0.018[a]	9.3[b]	0.018	410
(+)-Anatoxin-a	0.067	9.3	0.066	112
Leptodactyline	0.12	None	0.12	62
Anabaseine	0.74	NA	0.25	30
Acetylcholine	0.53	None	0.53	14
(S)-Nicotine	1.96	7.9	1.63	4.5
Cytisine	6.70	7.9[g]	5.56	1.3
Carbamylcholine	7.38	None	7.38	1.0
(S)-Anabasine	7.05	8.7	6.83	0.93

In the column on the far right, the potencies are calculated assuming that the mono-cationic (I) form of each compound is solely active. The median effective concentration (EC_{50}) values are calculated for a pH of 7.2. Adapted with permission from ref. *18*.

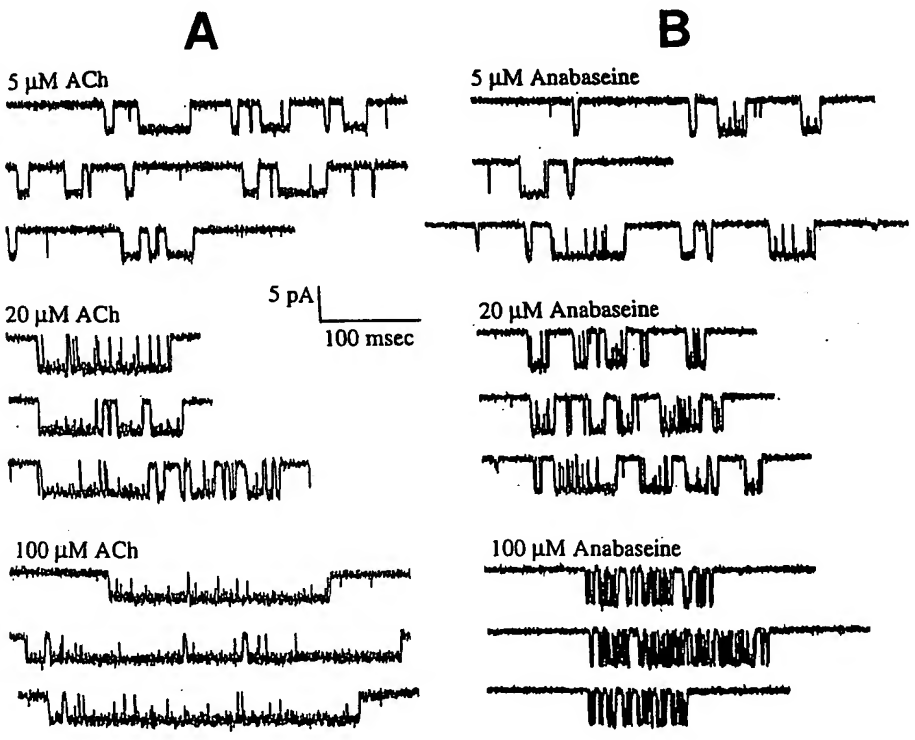


Fig. 3. Nicotinic agonist activity of anabaseine and ACh on BC3H-1 cells. Data were recorded using the cell-attached voltage-clamp method. Groupings of openings activated by either ACh (**A**) or anabaseine (**B**) are shown. Groups for analysis were selected as defined in the Methods. Qualitatively, closed intervals within groups of openings become shorter with increases in agonist concentration. With anabaseine, open intervals become shorter with increases in agonist concentration as a result of channel block by anabaseine, and an increase in frequency of a short duration gap is apparent. Adapted with permission from ref. *18*.

A

Normalized Response

B

Normalized Response

Fig. 4. Agonist
expressed in *Xenop*
relatively low effic
receptor. On this
anabaseine or anaba
to a control ACh (
point represents the
from ref. *18*.

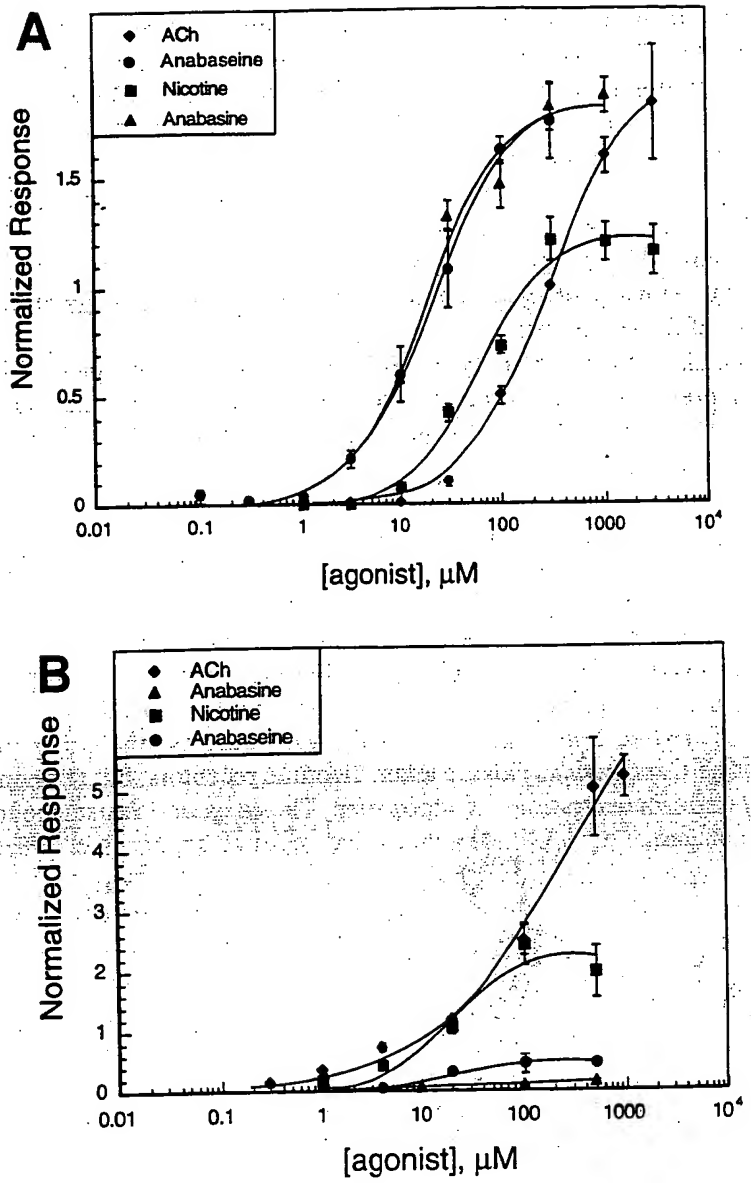


Fig. 4. Agonist actions of anabaseine and anabasine on rat brain nicotinic receptors expressed in *Xenopus* oocytes. **(A)** Responsiveness of the α_7 receptor. Note that nicotine has a relatively low efficacy for stimulating this receptor subtype. **(B)** Responsiveness of the α_4-β_2 receptor. On this receptor nicotine is a much more potent and efficaceous agonist than anabaseine or anabasine. Agonist responses were normalized to the individual oocyte's response to a control ACh (500 μM) application made 5 min before the compound application. Each point represents the average response (± S.E.) of at least four oocytes. Adapted with permission from ref. *18*.

of the rat brain, anabaseine elicited the same prostration behavior as many other nicotinic agonists *(18)*. The significantly lower potency of anabaseine, relative to nicotine, in causing prostration is consistent with the notion that β_2 subunit-containing nicotinic receptors primarily mediate this behavior. In the rat fronto-parietal cortex, anabaseine elevated both norepinephrine and acetylcholine levels without affecting serotonin and dopamine *(21)*. The noncompetitive nicotinic antagonist mecamylamine inhibited the anabaseine elevation of these two neurotransmitters (Fig. 5). These central anabaseine actions were most likely mediated through high-affinity nicotinic receptors containing beta2 subunits, whose channels are much more sensitive to mecamylamine blockade than are those of the α_7 receptors.

Anabaseine also affects a variety of invertebrate nicotinic receptors. Marine annelids, the usual prey of *Paranemertes,* are paralyzed, as are crustaceans and insects; it is assumed that these responses result from stimulatory actions upon nicotinic receptors. In these organisms nicotinic cholinergic receptors primarily reside on central neurons without readily recognizable soma, which makes experimental analysis of toxin effects more difficult. 2,3'-bipyridyl, a largely nonionized analog of anabaseine, is even more active than anabaseine in paralyzing crustaceans *(6)*. While it does not cause paralysis, nemertelline (a tetrapyridyl found in *Amphiporus angulatus*), in common with anabaseine and 2,3'-bipyridyl, stimulates an unusual receptor in the stomatogastric muscle of the crayfish, which is apparently a chloride channel *(22)*. At present this is the only known action of this complex alkaloid, which is the most abundant pyridine in this species of *Amphiporus*. Nemertine body-wall muscles (including those of the heteronemertine *Cerebratulus*) also contain nicotinic receptors, but they only respond to extremely high concentrations of anabaseine. Thus a natural resistance to this toxin may be advantageous to hoplonemertines that produce anabaseine or related compounds *(23)*.

Anabaseine also affects molluscan ganglionic nicotinic receptors, some of which are chloride channels. The ganglionic receptors are of three major types: chloride channels that either rapidly or slowly desensitize and cation channels that desensitize rather slowly. While anabaseine primarily blocks the very fast desensitizing receptor-chloride channel, it activates the sustained chloride channel response as well as the cation channel. In contrast, the anabaseine derivative DMXB anabaseine transiently activates and subsequently blocks the rapidly desensitizing chloride channel, at concentrations that do not affect the slowly inactivating chloride and cation channels. This pattern is consistent with its selective agonistic action on α_7 nicotinic receptors in vertebrates.

A variety of pyridine compounds including anabaseine and 2,3'-bipyridyl (*see* Subheading 2.3.) stimulate chemoreceptor neurons in crayfish and spiny lobster walking legs (*25*; Hatt, Ache, and Kem, unpublished results). Observations of feeding behavior in marine aquaria indicated that spiny lobsters attack but subsequently reject living *Amphiporus angulatus*. Anabaseine and 2,3'-bipyridyl were found to be two of the most active compounds in stimulating similar pyridine receptors on spiny lobster antennule nerves *(22)*. We suspect that nemertine alkaloids, by acting upon these chemoreceptors, may act as repellants against certain predators.

2.2. *DMXB-Anabaseine (GTS-21): A Synthetic Anabaseine Drug Candidate*

While anabaseine is a broad spectrum nicotinic agonist, a variety of 3-substituted anabaseines have been found to possess greater nicotinic receptor selectivity (*21*; Kem,

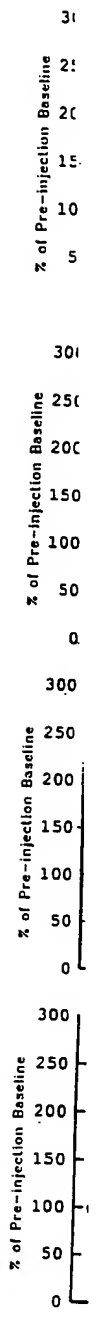


Fig. 5. Microdi
frontoparietal corte
ment (4.9 µmol/kg c
side).The s.c. dose
expressed as a perce
injection = 100%); r
with permission fro

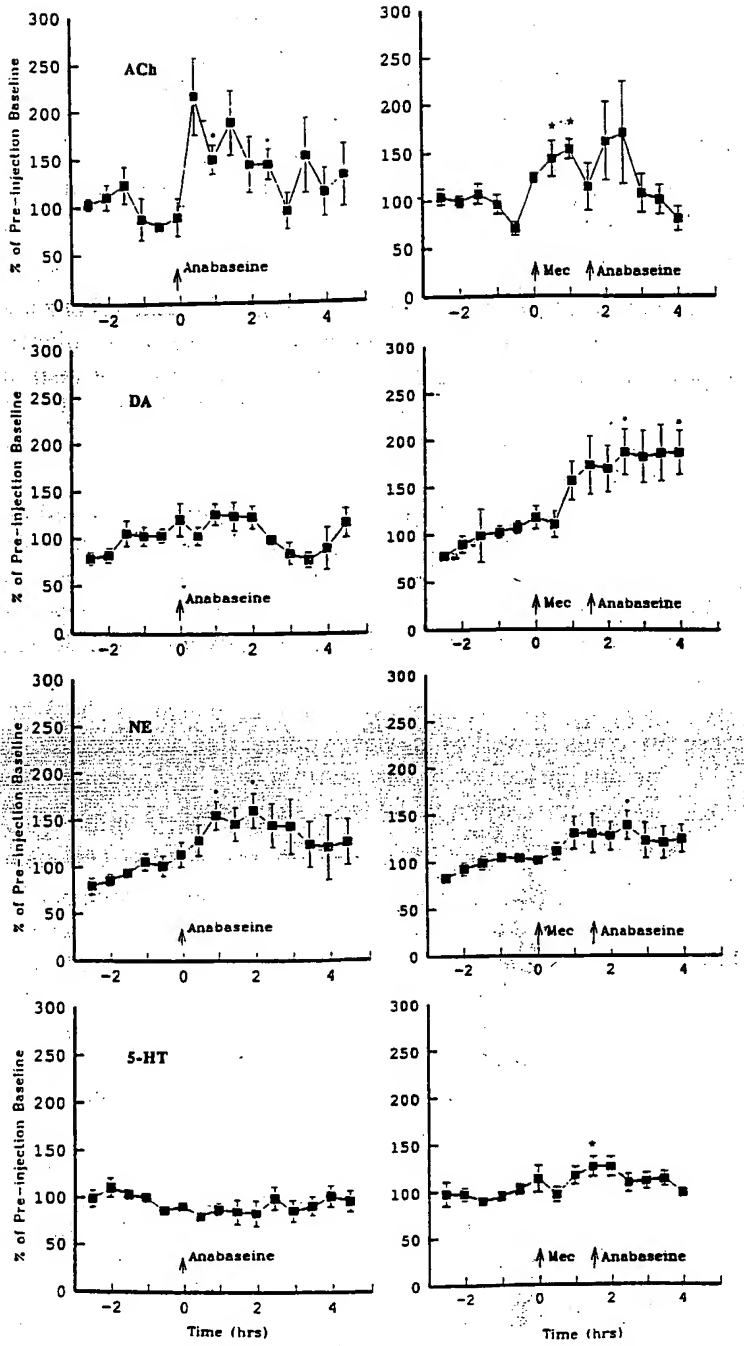


Fig. 5. Microdialysis study of anabaseine effects upon neurotransmitter levels in the rat frontoparietal cortex. Anabaseine was administered alone (*left side*) or 90 min after pretreatment (4.9 μmol/kg or 1.0 mg/kg, i.p.) with the noncompetitive antagonist mecamylamine (*right side*).The s.c. dose was 3.6 μmol/kg for each compound (0.90 mg/kg for anabaseine. Data are expressed as a percentage of the pre-injection control levels (average of the six samples prior to injection = 100%); mean ± S.E.M., $n = 6$, $^*p < 0.05$ by paired Students *t*-test analysis. Adapted with permission from ref. *21*.

W. R., et al., in preparation). Here we shall only consider 3-(2,4-dimethoxybenzylidene) -anabaseine, whose pharmaceutical code name is GTS-21 *(26–28)*. This compound is of special interest because it has been shown to be a neuroprotective agent in stroke and amino acid neurocytotoxicity models as well as a "cognition enhancer" in aged and brain lesioned animals. Initial (Phase I) tests in humans demonstrated the lack of toxicity of the compound and also indicate improved cognitive function in healthy young adults *(29)*.

2.2.1. *Chemistry*

GTS-21 (Fig. 2) is readily prepared by reaction of 2,4-dimethoxybenzaldehyde with anabaseine in acidic alcohol at elevated temperature, in a manner similar to the preparation of 3-(4-dimethylaminobenzylidene)-anabaseine *(13,30)*. The resulting product can be precipitated and recrystallized using less polar solvents. The 3-arylidene-anabaseines do not hydrolyze to open-chain forms at physiological pH like anabaseine, but they are moderately photolabile and must thus be protected from strong light. In principle, the benzylidene ring of such a compound can adopt two possible conformations with respect to the tetrahydropylridyl ringt, namely E (entegegen) or Z (zusamenfassung). By NMR we have shown that the E form is preferred in aqueous solution *(16)*. Only in the presence of intense light does the E to Z conversion become significant. The Z-form does not display significant affinity for the α_4-β_2 receptor (Kem et al., unpublished results).

While the two rings in anabaseine are essentially co-planar, in the 3-benzylidene-anabaseines these two rings as well as the benzylidene ring are predicted to lie in different planes *(30)*. Perhaps the lack of agonist activity of GTS-21 upon certain nicotinic receptor subtypes that are quite sensitive to anabaseine, such as α_4-β_2 and muscle

2.2.2. *Pharmacology*

In contrast to anabaseine, DMXB-anabaseine is only agonistic upon one known nicotinic receptor subtype, the neuronal homo-oligomeric α_7 receptor, which is primarily found in the brain. It also acts as an antagonist at the brain α_4-β_2 nicotinic receptor. Only at much higher concentrations does it act as a weak antagonist at other peripherally located nicotinic receptors. What makes this compound of considerable interest is its selective stimulation of a central nervous system (CNS) receptor whose physiological function has been very difficult to investigate in the past. Initially the α_7 receptor could only be recognized by its ability to bind α-bungarotoxin (BTX). Only later, after cloning and expression in cultured cells, was it found to be physiologically active as a ligand-gated ion channel with high permeability for calcium ions. The compound is a partial agonist at this receptor, since it displays an efficacy approximately half that of ACh for stimulating this receptor when expressed in the *Xenopus* oocyte (Fig. 6). Another cinnamylidene-anabaseine derivative, DMAC-anabaseine, is about as efficaceous as ACh *(27)*.

2.2.3. *Behavioral Effects*

It has now been over a decade since several laboratories reported large (as much as 50%) decreases in nicotinic cholinergic receptors in Alzheimer's patients *(31)*. This stimulated considerable academic and pharmaceutical interest in the development of nicotinic agonists that could selectively stimulate the remaining brain nicotinic receptors involved in cognitive and other critical mental functions. Its effects upon cognitive

Fig. 6. Comp
action on the rat
oocyte to 500 μM
sotfware. Each p
compounds) or th

behavior have b
mals. Initially it
mance in rats (
conditioned eye-
such as water- ;
monkeys *(35)* w
to enhance cogr
pound is current

DMXB-anaba
in mice *(36)*. Sin
of BTX, α_7 rec
antagonist at mo
nicotinic receptc

2.2.4. *Neuroprot*

DMXB-anaba
mocytoma (PC1
primary cultures
be mediated thro
or mecamylamir
rats *(42,43)*. Thu
well as ameliora
Alzheimer's.

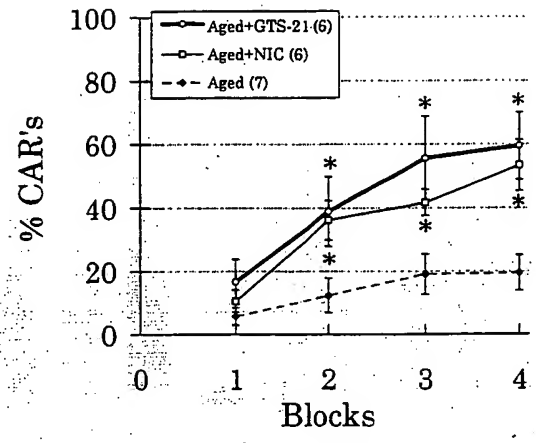


Fig. 6. Comparison of the effects of DMXB-anabaseine and DMAC-anabaseine with ACh action on the rat α_7 nicotinic receptor. Responses were normalized to the responsiveness of the oocyte to 500 μ*M* ACh applied 5 min earlier. The curves were obtained with Kaleidograph sotfware. Each point represents the mean ± 1SE of the response from four oocytes (anabaseine compounds) or three oocytes (Ach). Adapted with permission from ref. *27*.

behavior have been investigated by four different laboratories using a variety of mammals. Initially it was observed that the compound enhanced passive avoidance performance in rats *(20)*, active avoidance (Fig. 7) in aged rats *(32)*, and acquisition of conditioned eye-blink reflex (Fig. 8) in aging rabbits *(33)*. More intricate learning tasks such as water- and radial-maze performance by rats *(34)* and delayed matching by monkeys *(35)* were also enhanced, which suggests that the compound may also be able to enhance cognition in aging humans, particularly Alzheimer's patients. The compound is currently in clinical trials.

DMXB-anabaseine, like nicotine, has been demonstrated to enhance auditory gating in mice *(36)*. Since this action of both compounds is prevented by prior administration of BTX, α_7 receptors probably mediate this action. It is also a moderately potent antagonist at mouse 5-HT_3 receptors, which display a high degree of homology with α_7 nicotinic receptors *(37)*.

2.2.4. Neuroprotection

DMXB-anabaseine displays neuroprotective actions upon differentiated pheochromocytoma (PC12) cells stressed by nerve growth-factor depletion *(38)*, and neocortical primary cultures exposed to glutamate *(39,40)* or ß-amyloid *(41)*. This action seems to be mediated through α_7 nicotinic receptors since it can be inhibited by α-bungarotoxin or mecamylamine. Neuroprotection by DMXB-anabaseine has also been observed in rats *(42,43)*. Thus, this drug candidate could delay the process of neurodegeneration as well as ameliorate some of the cognitive deficits in neurodegenerative diseases like Alzheimer's.

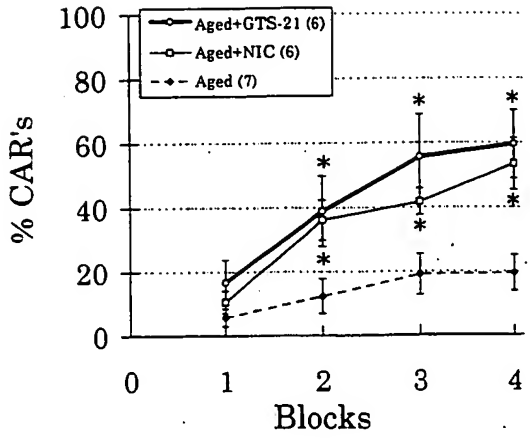


Fig. 7. Enhancement of one-way active avoidance acquisition in aged Sprague-Dawley rats pretreated with GTS-21 (1 mg/kg), nicotine (NIC; 0.2 mg/kg) or saline vehicle 15 min prior to daily testing for 12 d. The number of animals in each group is indicated in parentheses. Asterisks at individual 3-d time blocks indicate a significantly greater percentage of conditioned avoidance responses (CARs) for that group compared to the % CAR's exhibited by aged control rats for that block ($p < 0.05$ or higher level of significance). Adapted with permission from ref. *32*.

2.3. *Bipyridyl and Other Hoplonemertine Alkaloids*

The Chevron Nemertine (*Amphiporus angulatus*) is relatively common in the cold coastal waters of both the Atlantic and Pacific coasts of North America. It produces a wide variety of pyridyl alkaloids *(6,7,22)*. Anabaseine is only a relatively minor constituent in this species, which feeds upon crustaceans, in contrast with the anabaseine-rich, vermiferous *Paranemertes peregrina*. The major alkaloids are 2,3'-bipyridyl and a tetrapyridyl alkaloid named nemertelline (Fig. 9). While the crustacean paralyzing activity of the former compound is even greater than that of anabaseine, nemertelline lacks an obvious toxic activity upon mammals (mice) as well as crustaceans. In addition to these compounds we have recently identified a new dihydroisoquinoline alkaloid and an isomer of anabaseine, 2-(3-pyridyl)-1,2,5,6-tetrahydropyridine (Soti and Kem, in preparation). Many other compounds are present in smaller amounts. Since some of these compounds may be intermediates in the biosynthesis of the most abundant compounds, knowledge of their structures may assist in constructing potential biosynthetic pathways for these alkaloids.

The crustacean paralyzing activity of 2,3'-bipyridyl is particularly interesting because this molecule apparently acts in its nonionized form in this group of invertebrates. Since its most basic nitrogen pKa is 4.4, only about 1 in 10,000 molecules will exist as monocations at physiological pH *(44)*. This leads one to ask whether this substance acts upon nicotinic receptors, since all known forms of this receptor so far studied, invertebrate as well as vertebrate, seem to require that a stimulatory molecule contains a cationic group. Because crustacean nicotinic receptors are centrally located and radioligand receptor-binding investigations have yet to be done on these receptors, it is still only an assumption that this bipyridyl is acting on such receptors. Since 2,3'-bipyridyl also acts as a feeding repellent for spiny lobsters when incorporated into agar

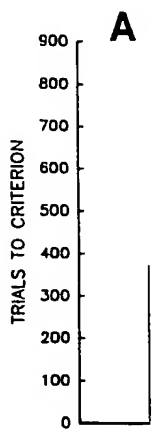


Fig. 8. Enh
upregulation by
to learn the ey
saline control g
concentration o
bits. No statisti
any of the GTS-
ref. *33*.

gel blocks con
a defensive to
It is highly
prey. One pos
tric-mill appar
ion-permeable
linergic agonis
bipyridyl and
breaks up inge
are planned, n

3. HETERON

Many heter
rotoxins that h
species, *Cereb*
have been fou

3.1. *Cerebrat*

3.1.1. *Chemist*

The so-call
similar to the s
The sequences
were reported

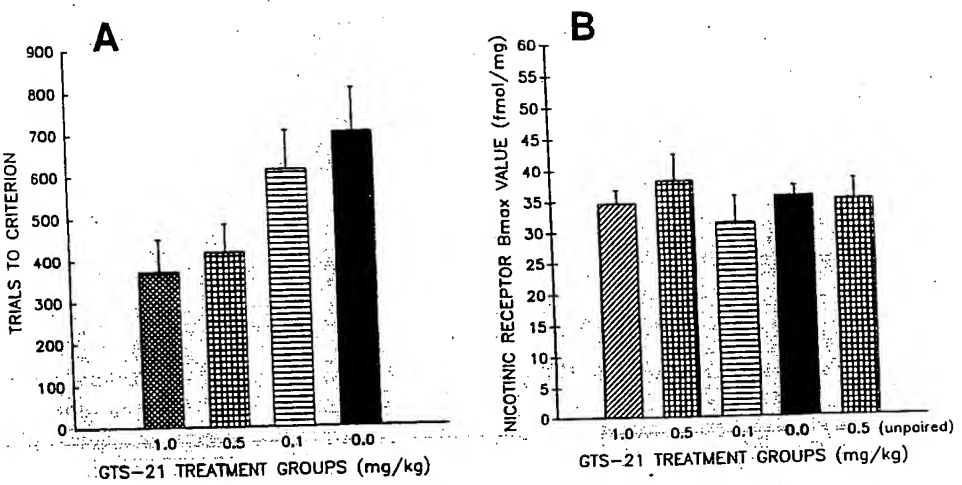


Fig. 8. Enhancement of eyeblink conditioning in aged rabbits and lack of nicotinic receptor upregulation by DMXBA. **(A)** on the left indicates the number of paired stimulus trials required to learn the eyeblink reflex at three different doses (subcutaneous) of GTS-21, relative to a saline control group ($n = 8$ animals for each group). **(B)** on the right shows measurements of the concentration of high nicotine affinity receptors in cerebral-cortex samples from the same rabbits. No statistically significant difference in receptor concentrations were observed between any of the GTS-21 treated animal groups and the control group. Adapted with permission from ref. *33*.

gel blocks containing a feeding attractant, it may serve as a feeding deterrent as well as a defensive toxin.

It is highly likely that nemertelline also has some action on potential predators or prey. One possible site of its action would be the skeletal muscles composing the gastric-mill apparatus in the crustacean stomach. These skeletal muscles possess chloride ion-permeable ion channels that are activated by most commonly used nicotinic cholinergic agonists. Prolonged activation of these depolarizing chloride currents by 2,3'-bipyridyl and nemertelline would be expected to block the initial grinding process that breaks up ingested organisms and other food materials. Further studies of nemertelline are planned, now that a method of laboratory synthesis has been worked out *(45)*.

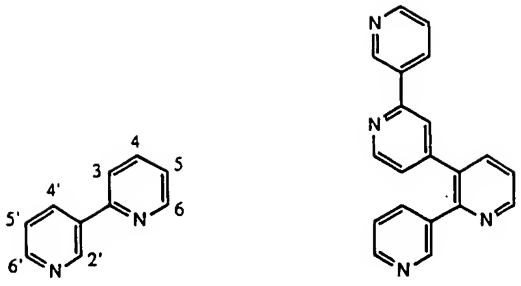
3. HETERONEMERTINE NEUROTOXINS

Many heteronemertines possess peptide neurotoxins *(8,9)*. However, the only neurotoxins that have been isolated to date belong to a large (>1 m, 20 g) Atlantic coast species, *Cerebratulus lacteus*. The other group of anoplans, paleonemertines, thus far have been found to contain only protein cytolysins (Kem, unpublished results).

3.1. *Cerebratulus* Neurotoxins

3.1.1. Chemistry

The so-called *Cerebratulus* B neurotoxins have molecular sizes of approx 6000, similar to the scorpion neurotoxins, and are crosslinked by three disulfide bonds *(46)*. The sequences (Fig. 10) of the two most abundant and active isotoxins, B-II and B-IV, were reported some time ago *(47,48)*. Both are very basic peptides and contain a single



2,3'-Bipyridine Nemertelline

Fig. 9. Molecular structures of the major pyridyl alkaloids of the hoplonemertine *Amphiporus angulatus*. 2,3'-Bipyridyl (*left side*) is the major neurotoxin of this nemertine, while the most abundant alkaloid is nemertelline (*right side*). Nemertelline affects crustacean stomach muscle nicotinic receptors but is not toxic (Kem and Soti, submitted). The structure of nemertelline was recently revised *(45)*.

residue of hydroxyproline at position 10. Unlike the scorpion and sea anemone peptide toxins whose secondary structures are largely composed of anti-parallel B-strands, the B toxins are devoid of B-sheet structure but rich in α-helix *(49)*. The secondary and tertiary structures of B-IV were determined by NMR *(50,51)*. It can be observed that there are two long stretches of helix, represented by positions 11-23 and 34-49. The two helices are connected by a loop consisting of two inverse γ-turns and a ß-turn. The entire sequence, 11-49, thus constitutes a rather unique helical hairpin structure (Fig. 11).

3.1.2. Pharmacology

The two B toxins are potent toxins when injected into crustaceans, especially crayfish. Initially the animal displays tremors (including flipping of the tail), but then convulses in a massive contracture of the limbs and tail. In a few minutes the contractural paralysis is replaced by flaccid paralysis and eventually death. This toxicity of the *Cerebratulus* B toxins (and unpurified *Lineus* toxins) seems confined to the crustacean nervous system. The limb contracture can also be observed in the perfused crayfish cheliped. The nerve terminals seem to be the site of action, since tetrodotoxin effectively blocks the action of toxin B-IV, even when the peptide is applied directly on the muscle. At relatively high (0.1–1.0 μ*M*) concentrations toxin B-IV also affects the evoked compound action potential recorded from isolated crab-leg nerves, causing some repetitive spiking, which also probably contributes to the prolongation of the compound action potential (Kem, W. R., unpublished results). The current conception of how the toxins act is that they activate a small population of sodium channels, which leads to neuronal repetititive spiking, which causes a massive release of excitatory (and perhaps inhibitory) neurotransmitters at neuromuscular synapes. In arthropods the skeletal muscles are generally electrically inexcitable, but are innervated along their entire length by excitatory glutamatergic and inhibitory GABAergic synapes. A more intensive analysis of their actions using intracellular or patch clamp recording techniques would certainly be desirable. B-IV was inactive when tested on a variety of isolated axons and neurons from various noncrustacean groups including vertebrates and molluscs *(46)*.

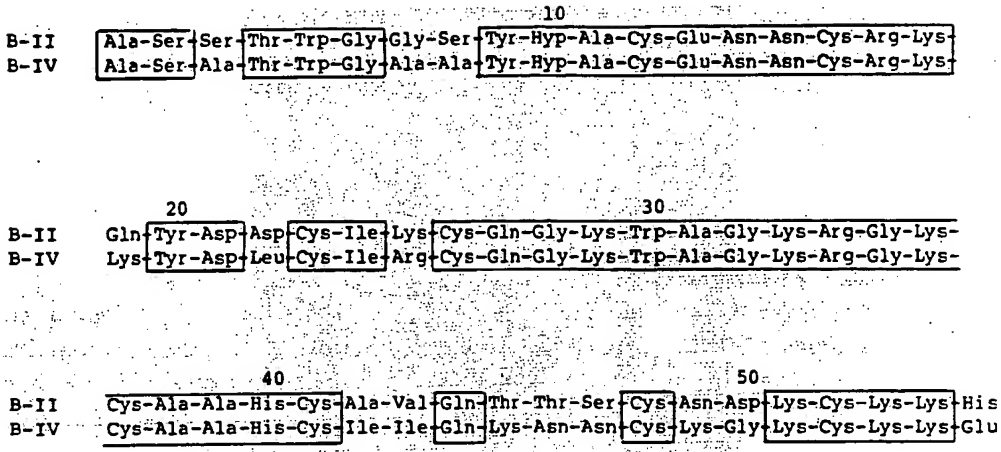


Fig. 10. Amino acid sequences of the two *Cerebratulus lacteus* neurotoxins B-II and B-IV. Identical sequence regions are enclosed within boxes. Adapted with permission from refs. *47* and *48*.

3.1.3. Receptor Binding Studies

Lieberman and Blumenthal *(52)* measured the binding of iodinated toxin B-IV to membranes prepared from Maine lobster (*Homarus vulgaris*) muscle. High-affinity binding was observed. The specific binding could not be displaced by scorpion α-toxin. Further experiments are required to identify the membrane receptor involved. While they suceeded in chemically crosslinking the toxin to the membranes, the solubilized membrane proteins labeled by the iodinated toxin possessed a smaller molecular size (about 40,000) than would be predicted for the sodium channel α-subunit, which contains the binding site for scorpion and sea anemone peptide toxins. This result could be explained in several ways. Membrane proteins can be degraded by endogenous proteases unless a cocktail of inhibitors was added. This seems unlikely to be the reason here, since several inhibitors were added to the incubation saline. Other alternative explanations are that the toxin either binds predominantly to a B subunit of the sodium channel or that it interacts with some other membrane protein, perhaps another ion channel.

3.1.4. Structure-Action Relationships

Considerable data is available implicating some amino acid sidechains in the toxic action of toxin B-IV. The initial studies utilized a chemical modification approach and focused on the few aromatic residues (2 tyrosyls, 2 tryptophanyls). By manipulating the conditions of the reactions, it was possible to differentially label the two Tyr and two Trp residues, the former by nitration *(53)* and the latter by alkylation *(54)*. By bioassaying the toxin samples at different degrees of modification, it was deduced that Tyr9 and Trp30 are probably involved in receptor binding, since their modification did not affect secondary structure as measured by CD spectroscopy.

During the past decade Blumenthal's lab has utilized molecular biological methods to express B-IV in *Escherichia coli* and to obtain mutants for structure-activity relationship (SAR) studies *(55,56)*. Initial experiments showed that replacement of the

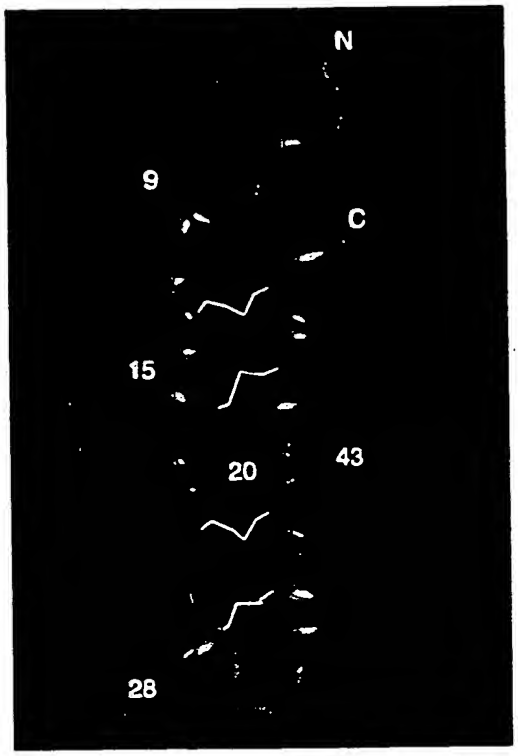


Fig. 11. An NMR-derived solution backbone structure of *Cerebratulus* neurotoxin B-IV. Practically the entire peptide consists of a helical hairpin structure crosslinked by four disulfide bonds. Adapted with permission from ref. *51*.

hydroxyproline at position 10 with Pro did not affect crayfish paralytic activity, nor did replacement of Ala residues at either position 3 or 8 with serine. In fact, the toxicity of B-IV was enhanced by simultaneous substitution of serine at positions 3 and 8. Arg17 has been implicated in receptor binding as toxicity was undetectable but the CD spectrum unchanged when glutamine, Ala or Lys was substituted. Replacement of Arg25 with Lys reduced crayfish toxicity 400-fold *(57)*. Some of the side chains implicated in toxicity of B-IV include the guanidinyl side chains of Args 17, 25, and 34, and the aromatic side chains of Trp 30 and Tyr 9. It was rather surprising that the implicated residues are found along the entire length of one surface of the toxin. This implies that the toxin binds to an extensive portion of receptor surface. Alternately, modification of certain residues such as Tyr9 and Trp30 may have altered the folded structure sufficiently to deleteriously affect activity without affecting the CD spectrum. Experiments with other toxin mutants, coupled with more intensive NMR structural analyses of tertiary structure should provide further insights regarding the binding surface of this toxin. Since a scorpion α-toxin did not inhibit the *Cerebratulus* toxin binding, the latter probably binds to another site *(52)*.

3.2. *Lineus Neurotoxins*

All species of this large heteronemertine genus so far examined have been found to be quite toxic *(8,58,59)*. These toxins are also low molecular weight peptides of 3000–

6000 Daltons *(4)*. While they paralyze crustaceans in a manner indistinguishable from the *Cerebratulus* toxins, the *Lineus* toxins primarily prolong action potential duration in crustacean neurons, whereas *Cerebratulus* toxin B-IV causes repetitive spiking. Isolation and characterization of the *Lineus* toxins is currently being attempted in the author's laboratory.

4. HETERONEMERTINE AND PALEONEMERTINE CYTOLYSINS

Lytic toxins are practically ubiquitous among all living organisms, including bacteria, plants, and animals. Almost all animal venoms include some lytic substances that enhance the penetration of the other toxins into the circulation of the affected organism and possibly potentiate the actions of certain toxins. So far, cytolytic proteins have only been found in the anoplan nemertines.

Cerebratulus contains at least four homologous protein lysins called A toxins *(60)*. The most abundant isotoxin, A-III, was sequenced and shown to contain three disulfide bonds *(61)*. CD and Raman spectroscopic analyses of this toxin revealed the presence of approx 60% α-helix and 10% B-sheet (Kem, W. R., et al., in preparation). The C-terminal portion that is not cross-linked by a disulfide bond is thought to exist as a helical hairpin structure. Because of its amphipathic nature, this region of toxin sequence may interact with membrane lipids and possibly participate in the formation of a pore.

At sublytic concentrations *Cerebratulus* toxin A-III blocks the squid axon sodium channel and affects the kinetics of opening and closing of voltage-gated potassium channels. Then it increases the so-called leakage conductance, which probably reflects a dimunition of membrane integrity *(58)*. These differential effects on sodium vs potassium channels are interesting and deserve further study. Perhaps due to its ability to disrupt lipid bilayers, toxin A-III also inhibits brain phospholipid sensitive Ca^{2+}-dependent protein kinase in vitro, as do other peptide toxins that possess a detergent-like action *(61)*. Also, these proteins may be neurotoxic at lower, sublytic concentrations and potentiate the actions of the smaller neurotoxic B peptide toxins.

5. CONCLUDING COMMENTS

It is predicted that systematic investigations of other nemertine species will reveal a plethora of molecules used as toxins by this relatively unstudied phylum. Besides providing unique tools for biomedical research, some of the toxins (particularly alkaloids like anabaseine) may become molecular models for drug design *(63,64)*. The toxins will also provide insights into chemical mechanisms and structures. For instance, the NMR investigation of anabaseine has provided an improved understanding of the stability of tetrahydropyridine compounds under aqueous conditions. Norton's NMR solution structural analysis of *Cerebraulus* toxin B-IV has revealed a paired helical structure that is so far unique among the known neurotoxins. Many invertebrate toxins are primarily directed towards other invertebrates; that is, they have evolved over time to deal with potential invertebrate predators or prey. Study of their actions upon invertebrate nervous system receptors may ultimately lead to the design of more selective pesticides and anti-parasitic drugs. Ultimately it is expected that homologous toxins will be found that also target homologous receptors in the mammalian nervous system. For

instance, although the narrow phylogenetic activity of the *Cerebratulus* B neurotoxins presently limits their utility as molecular probes to crustacean nervous systems, it seems likely that natural or synthetic variants of these toxins will eventually be found that act on homologous ion channels of mammals and other vertebrates.

ACKNOWLEDGMENTS

The author is grateful to many students, colleagues, and technical personnel for their contributions to this research, which has spanned the past three decades. Barbara Seymour prepared the drawings in Fig. 1. Many of the author's investigations of nemertine neurotoxins were supported by NSF, NIH, or Taiho Pharmaceutical Company grants.

REFERENCES

1. Bacq, Z. M. (1936) Les poisons des Nemertiens. *Bull. Acad. R. Belg. Cl. Sci.* (Ser. 5) **22:** 1072–1079.
2. Bacq, Z. M. (1937) L"amphiporine" et la "nemertine," poisons des vers nemertiens. *Arch. Int. Physiol.* **44,** 190–204.
3. King, H. (1939) Amphiporine, an active base from the marine worm *Amphiporus lactifloreus. J. Chem. Soc.* **1365.**
4. Kem, W. R. (1969) A chemical investigation of nemertine toxins. Ph.D. dissertation, University of Illinois, Urbana.
5. Kem, W. R., Abbott, B. C., and Coates, R. M. (1971) Isolation and structure of a hoplonemertine toxin. *Toxicon* **9,** 15–22.
6. Kem, W. R., Scott, K. N., and Duncan, J. H. (1976) Hoplonemertine worms: a new source of pyridine neurotoxins. *Experientia* **32,** 684–686.
7. Kem, W. R. (1988) Pyridine alkaloid distribution in the hoplonemertinea. *Hydrobiologia* **156,** 145–153.
8. Kem, W. R. (1971) A study of the occurrence of anabaseine in *Paranemertes* and other nemertines. Toxicon **9,** 23–32.
9. Kem, W. R. (1988) Worm toxins, in *Handbook of Natural Toxins,* vol. 4, *Marine Toxins and Venoms* (Tu, ed.), Marcel Dekker, New York, pp. 253–378.
10. Gibson, R. (1972) *Nemerteans.* Hutchinson University Library, London, pp. 224.
11. Wheeler, J. W., Olubajo, O., Storm, C. B., and Duffield, R. M. (1981) Anabaseine: venom alkaloid of *Aphaenogaster* ants. *Science* **211,** 1051–1052.
12. Spath, E. and Mamoli, L. (1936) Eine Neue Synthese Des D,L-Anabasins. *Chem. Ber.* **69,** 1082–1085.
13. Kem, W. R. (1973) Biochemistry of nemertine toxins, in *Marine Pharmacognosy: Marine Biotoxins as Probes of Cellular Function* (Martin, D. F. and Padilla, G. M., eds.), Monographs on Cell Biology Series, Academic Press, NY, pp. 37–84.
14. Bloom, L. B. (1990) Influence of solvent on the ring-chain hydrolysis equilibrium of anabaseine and synthesis of anabaseine and nicotine analogues. Ph.D. dissertation, University of Florida, Department of Chemistry.
15. Zoltewicz, J. A. and Cruskie, M. P., Jr. (1995a) A superior synthesis of cholinergic anabaseine. *OPPI Briefs* **27,** 510–513.
16. Zoltewicz, J. A., Bloom, L. B., and Kem, W. R. (1989) Quantitative determination of the ring-chain hydrolysis equilibrium constant for anabaseine and related tobacco alkaloids. *J. Org. Chem.* **54,** 4462–4468.
17. Kem, W. R., Mahnir, V. M., Bloom, L. B., and Gabrielson, B. J. (1994) The active form of the nicotinic receptor agonist anabaseine is the cyclic iminium cation. 11th World Congress on Animal, Plant, and Microbial Toxins, Tel Aviv.

18. Kem, W.
nist upon
Pharmac
18a. Gibson, R
19. Barlow, R
nicotine n
20. Meyer, E.
Effects of
ance beha
21. Summers,
rotransmit
22. Kem, W. F
sity: Initia
23. Kem, W. I
unique nic
Eilat, Israe
24. Kehoe, J.
alpha-bung
25. Hatt, H. an
units on th
Physiol. **15**
26. Kem, W. R
which is a
27. De Fiebre,
L. (1995) C
3-(4)-Dime
nicotinic al
164–171.
28. Kem, W. R.
target for th
Brain Res. 1
29. Kitagawa, H
R. (1998) P
measures of
30. Zoltewicz, J
transmission
lated by mol
31. Whitehouse,
Nicotinic ac
32. Arendash, G
and memory
21. *Brain Re.*
33. Woodruff-Pa
21), eyeblink
Res. **645,** 30
34. Bjugstad, K.
treatment wit
affecting the
19–28.
35. Briggs, C. A.,
E., et al. (199
receptor ligar

18. Kem, W. R., Mahnir, V. M., Papke, R., and Lingle, C. (1997) Anabaseine is a potent agonist upon muscle and neuronal alpha-bungarotoxin sensitive nicotinic receptors. *J. Pharmacol. Exper. Therap.* **283,** 979–992.
18a. Gibson, R. (1972) *Nemerteans*. Hutchinson University Library, London, pp. 224.
19. Barlow, R. B. and Hamilton, J. T. (1962) The effects of pH on the activity of nicotine and nicotine methiodide on the rat diaphragm preparation. *Br. J. Pharmacol.* **18,** 543–549.
20. Meyer, E. M., deFiebre, C. M., Hunter, B. E., Simpkins, C. E., and deFiebre N. E. (1994) Effects of anabaseine related analogs on rat brain nicotinic receptor binding and on avoidance behavior. *Drug Dev. Res.* **31,** 135–141.
21. Summers, K., Kem, W. R., and Giacobini, E. (1997) Nicotinic agonist modulation of neurotransmitter levels in the rat frontoparietal cortex. *Jpn. J. Pharmacol.* **74,** 139–146.
22. Kem, W. R. and Soti, F. (2001) *Amphiporus* alkaloid multiplicity implies functional diversity: Initial studies on crustacean pyridyl receptors. *Hydrobiolog.* In press.
23. Kem, W. R. (1997b) Nemertine body wall and proboscis longitudinal muscles possess unique nicotinic receptors. Fifth Intern. Conf. Invertebrate Neurochem. Neurophysiol. Mtg, Eilat, Israel (Abstr.).
24. Kehoe, J. S. and Kem, W. R. (2001) Anabaseine and GTS-21 act differentially on two alpha-bungarotoxin-sensitive *Aplysia* nicotinic receptors. *Toxicon,* in press.
25. Hatt, H. and Schmiedel-Jacob, I. (1984) Electrophysiological studies of pyridine-sensitive units on the crayfish walking leg. I. Characteristics of stimulatory molecules. *J. Comp. Physiol.* **154A,** 855–863.
26. Kem, W. R. (1998) Alzheimer's drug design based upon an invertebrate toxin (anabaseine) which is a potent nicotinic receptor antagonist. *Invertebr. Neurosci.* 3, 251–259.
27. De Fiebre, C. M., Meyer, E. M., Henry, J. C., Muraskin, S. I., Kem, W. R., and Papke, R. L. (1995) Characterization of a series of anabaseine-derived compounds reveals that the 3-(4)-Dimethylaminocinnamylidine derivative (DMAC) is a selective agonist at neuronal nicotinic alpha 7/[^{125}K] alpha-bungarotoxin receptor subtypes. *Mol. Pharmacol.* **47,** 164–171.
28. Kem, W. R. (2000) The brain alpha7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimer's disease: studies with DMXBA (GTS-21). Behav. Brain Res. **113,** 169–183.
29. Kitagawa, H., Moriyama, A. A., Takenouchi, T., Wesnes, K., Kramer, W., and Clody, D. R. (1998) Phase I studies of GTS-21 to assess the safety, tolerability, PK and effects on measures of cognitive function in normal volunteers. *Neurobiol. Aging* **19,** *S182* (Abstr.).
30. Zoltewicz, J. A., Prokai-Tatrai, K., Bloom, L. B., and Kem, W. R. (1993) Long range transmission of polar effects of cholinergic 3-arylideneanabaseines. Conformations calculated by molecular modelling. *Heterocycles* **35,** 171–179.
31. Whitehouse, R. J., Price, D. L., Clark, A. W., Coyle, J. T., and DeLong, M. R. (1986) Nicotinic acetylcholine binding in Alzheimer's disease. *Brain Res.* 371, 146–151.
32. Arendash, G. W., Sengstock, G. J., Sanberg, R., and Kem, W. R. (1995) Improved learning and memory in aged rats with chronic administration of the nicotinic receptor agonist GTS-21. *Brain Res.* **674,** 252–259.
33. Woodruff-Pak, D. S., Li, Y.-T., and Kem, W. R. (1994) A nicotinic receptor agonist (GTS-21), eyeblink classical conditioning, and nicotinic receptor binding in rabbit brain. *Brain Res.* **645,** 309–317.
34. Bjugstad, K. B., Mahnir, V. M., Kem, W. R., and Arendash, G. W. (1996) Long-term treatment with GTS-21 or nicotine enhances water maze performance in aged rats without affecting the density of nicotinic receptor subtypes in neocortex. *Drug Dev. Res.* **39,** 19–28.
35. Briggs, C. A., Anderson, D. J., Brioni, J. D., Buccafusco, J. J., Buckley, M. J., Campbell, J. E., et al. (1997) Functional characterization of the novel neuronal nicotinic acetylcholine receptor ligand GTS-21 *in vitro* and *in vivo*. *Pharmacol. Biochem. Behav.* **57,** 231–241.

36. Stevens, K. E., Kem, W. R., Mahnir, V. M., and Freedman, R. (1998) Selective alpha7-nicotinic agonists normalize inhibition of auditory response in DBA mice. *Psychopharmacology* **136,** 320–327.
37. Machu, T. K., Strahlendorf, J., and Kem, W. R. (1996) Nicotinic receptor ligands antagonize 5-HT_3 receptors expressed in *Xenopus* oocytes. *J. Neurosci.* **22,** 1780 (Abstr.).
38. Martin, E. J., Panickar, K. S., King, M. A., Deyrup, M., Hunter, B. E., Wang, G., and Meyer, E. M. (1994) Cytoprotective actions of 2,4-dimethoxybenzylidene anabaseine in differentiated PC12 cells and septal cholinergic neurons. *Drug Dev. Res.* **31,** 135–141.
39. Akaike, A., Tamura, Y., Yokota, T., Shimohama, S., and Kimura, J. (1994) Nicotine-induced protection of cultured cortical neurons against N-methyl-D-aspartate recetpor-mediated glutamate cytotoxicity. *Brain Res.* **644,** 181–187.
40. Shimohama, S., Greenwald, D. L., Shafron, D. H., Akaika, A., Maeda, T., Kaneko, S., et al. (1998) Nicotinic alpha7 receptors protect against glutamate neurotoxicity and neuronal ischemic damage. *Brain Res.* **779,** 359–363.
41. Kihara, T., Shmohama, S., Sawada, H., Kimura, J., Kume, T., Kochiyama, H., et al. (1997) Nicotinic receptor stimulation protects neurons against B-amyloid toxicity. *Ann. Neurol.* **42,** 159–163.
42. Nanri, M., Yamamoto, J., Miyake, H., and Watanabe, H. (1998) Protective effect of GTS-21, a novel nicotinic receptor agonist, on delayed neuronal death induced by ischemia in gerbils. *Jpn. J. Pharmacol.* **76,** 23–29.
43. Meyer, E. M., King, M. A., and Meyers, C. (1998) Neuroprotective effects of 2,4-dimethoxybenzylidene anabaseine (DMXB) and tetrahydroaminoacridine (THA) in nerocortices of nucleus basalis lesioned rats. *Brain Res.* **786,** 252–254.
44. Yamamoto, I., Kamimura, H., Yamamoto, R., Skai, S., and Goda, M. (1962) Studies on nicotinoids as insecticides. I. Relation of structure to toxicity. *Agr. Biol. Chem. (Tokyo)* **26,** 709–716.
45. Zoltewicz, J. A. and Cruskie, M. P., Jr. (1995b) Strategies for the synthesis of unsymmetrical quaterpyridines using palladium-catalyzed cross-coupling reactions. *Tetrahedron* **51,** 11,393–11,400.
46. Kem, W. R. (1976) Purification and characterization of a new family of polypeptide neurotoxins from the heteronemertine *Cerebratulus lacteus* (Leidy). *J. Biol. Chem.* **251,** 4184–4192.
47. Blumenthal, K. M. and Kem, W. R. (1976) Primary structure of *Cerebratulus lacteus* toxin B-IV. *J. Biol. Chem.* **251,** 6025–6029.
48. Blumenthal, K. M., Keim, P. S., Heinrikson, R. L., and Kem, W. R. (1981) Structure and action of heteronemertine polypeptide toxins. Amino acid sequence of *Cerebratulus lacteus* toxin B-II and revised structure of toxin B-IV. *J. Biol. Chem.* **256,** 9063–9067.
49. Kem, W. R., Tu, C.-K., Williams, R. W., Toumadje, A., and Johnson, W. C., Jr. (1990) Circular dichroism and laser Raman spectroscopic analysis of the secondary structure of *Cerebratulus lacteus* toxin B-IV. *J. Prot. Chem.* **9,** 433–443.
50. Hansen, P. E., Kem, W. R., Bieber, A. L., and Norton, R. S. (1992) ^{1}H-NMR study of neurotoxin B-IV from the marine worm *Cerebratulus lacteus*. Solution properties, sequence-specific resonance assignments, secondary structure and global fold. *Eur. J. Biochem.* **210,** 231–240.
51. Barnham, K. J., Dyke, T. R., Kem, W. R., and Norton, R. S. (1997) Structure of neurotoxin B-IV from the marine worm *Cerebratulus lacteus*: a helical hairpin cross-linked by disulphide bonding. *J. Mol. Biol.* **268,** 886–902.
52. Lieberman, D. L. and Blumenthal, K. M. (1986) Structure and action of heteronemertine polypeptide toxins. Specific cross-linking of *Cerebratulus lacteus* toxin B-IV to lobster axon memebrane vesicles. *Biochim. Biophys. Acta* **855,** 1–48.
53. Blumenthal, K. M. and Kem, W. R. (1980) Inactivation of *Cerebratulus lacteus* toxin B-IV by tyrosine nitration. *Arch. Biochem. Biophys.* 203,816–203,821.

54. Blumenthal, I
with tryptoph
55. Howell, M. L
for *Cerebratu*
56. Howell, M. L.
toxin B-IV id
Chem. **266,** 1
57. Wen, P. H. an
potency of neu
58. Kem, W. R.
Cerebratulus
59. Kem, W. R. (1
60. Kem, W. R. a
lytic *Cerebrat*
61. Blumenthal, K
A-III. *J. Biol.*
62. Kuo, J. F., Ray
(1983) Cobra
potent and sel
FEBS Lett. **153**
63. Kem, W. R. (2
ins of Animal

54. Blumenthal, K. M. (1980) Inactivation of *Cerebratulus lacteus* toxin B-IV concomitant with tryptophan alkylation. *Arch. Biochem. Biophys.* **203,** 822–826.
55. Howell, M. L. and Blumenthal, K. M. (1989) Cloning and expression of a synthetic gene for *Cerebratulus lacteus* neurotoxin B-IV. *J. Biol. Chem.* **264,** 15,268–15,273.
56. Howell, M. L. and Blumenthal, K. M. (1991) Mutagenesis of *Cerebratulus lacteus* neurotoxin B-IV identifies NH_2-terminal sequences important for biological activity. *J. Biol. Chem.* **266,** 12,884–12,888.
57. Wen, P. H. and Blumenthal, K. M. (1996) Role of electrostatic interactions in defining the potency of neurotoxin B-IV from *Cerebratulus lacteus*. *J. Biol. Chem.* **271,** 29,752–29,758.
58. Kem, W. R. (1994) Structure and membrane actions of a marine worm cytolysin, *Cerebratulus* toxin A-III. *Toxicol* **87,** 189–203.
59. Kem, W. R. (1985) Structure and action of nemertine toxins. *Am. Zool.* **2,** 99–111.
60. Kem, W. R. and Blumenthal, K. M. (1978) Purification and characterization of the cytolytic *Cerebratulus* A toxins. *J. Biol. Chem.* **253,** 5752–5757.
61. Blumenthal, K. M. and Kem, W. R. (1980) Primary structure of *Cerebratulus lacteus* toxin A-III. *J. Biol. Chem.* **255,** 8266–8272.
62. Kuo, J. F., Raynor, R. L., Mazzei, G. J., Schatzman, R. C., Turner, R. S., and Kem, W. R. (1983) Cobra polypeptide cytotoxin I and marine worm polypeptide cytotoxin A-IV are potent and selective inhibitors of phospholipid sensitive Ca^{2+}-dependent protein kinase. *FEBS Lett.* **153,** 183–186.
63. Kem, W. R. (2000), Nemertine neurotoxins, in Neurotoxicology Handbook: Natural Toxins of Animal Origin (Harvey, A., ed.), Humana Press, Totowa, NJ.